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Auf den Bescheid vom 8. März 2004

1. Ansprüche

Hiermit wird mit einer Ersatzseite ein neuer Anspruch 4 eingereicht. Das Merkmal "antibakterielles Peptid oder Protein" ist gestützt auf Seite 2, Zeile 18 sowie Seite 5, 1. Absatz der Beschreibung.

2. Neuheit

Anspruch 1 ist neu gegenüber D4.

D4 offenbart weder einen Transportvermittler, der die prokaryontische Zellmembran passiert, noch eine Peptid-Nukleinsäure (PNA), die die Transkription eines prokaryontischen Gens hemmt.

Das in D4 auf Seite 4 beschriebene bakterielle Transportmolekül ist ein zum

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Penetratin (Seite 3, letzter Absatz) homologes Peptid, das aus E. coli isoliert wurde, wovon sich die Bezeichnung "bakteriell" herleitet. Die drei in D4 spezifisch beschriebenen Transportvermittler können jedoch die bakterielle Zellwand nicht überwinden und sind deshalb nicht als Carrier-Moleküle für den Transport in Prokaryonten geeignet.

Ferner offenbart D4 keine PNA, die gegen ein prokaryontisches Gen gerichtet ist. Das in Beispiel 4 von D4 beschriebene c-myc Gen ist ein Onkogen viralen Ursprungs, das nur in Wirbeltieren vorhanden ist (siehe Ann. Rev. Genet. 1986. 20, Seiten 362-363). Folglich ist c-myc kein prokaryontisches Gen.

3. Erfinderische Tätigkeit

D4 beschreibt ein Konjugat zum Transport von PNA in eukaryontische Zellen. Wie oben ausgeführt, werden in D4 weder Transportvermittler, die die prokaryontische Zellwand passieren, noch eine PNA, die gegen ein prokaryontisches Gen gerichtet ist, offenbart.

Auch die in D8 beschriebenen Studien zur Hemmung der Transkription von Genen betreffen ausschließlich eukaryontische Gene.

Weder D4 noch D8 enthalten einen Hinweis auf die Hemmung der Transkription von prokaryontischen Genen. Folglich wird Anspruch 1 weder durch D4 noch durch die Kombination aus D4 und D8 nahegelegt.

Außerdem beschreibt die Erfindung ein neues Therapiekonzept, das von bisherigen Strategien abweicht. Die erfindungsgemäßen Konjugate erzielen ihre besondere Effizienz auch durch die, insbesondere in den Ansprüchen 4, 5 und 6 beanspruchte, Doppelstrategie, bei der antibakterielle Peptide mit einer gegen ein prokaryontisches Gen gerichteten PNA konjugiert werden.

Patentanwalt

Dr. Andrea Schüßler

Anlage:
-Ersatzseite mit geänderten Anspruch 4
-Ann. Rev. Genet. (1986) 20:361-84

Geänderte Patentansprüche

1. Konjugat, das zur Behandlung prokaryontischer Infektionen geeignet ist und die folgenden Komponenten aufweist:

(a) einen die prokaryontische Zellmembran passierenden Transportvermittler; und

(b) eine in den Prokaryonten einzubringende und gegen diese gerichtete Peptid-Nukleinsäure (PNA), die die Transkription eines prokaryontischen Gens hemmt.

2. Konjugat nach Anspruch 1, wobei der Prokaryont ein Bakterium ist.

3. Konjugat nach Anspruch 2, wobei das Bakterium ein humanpathogenes Bakterium ist.

4. Konjugat nach einem der Ansprüche 1 bis 3, wobei der Transportvermittler ein antibakterielles Peptid oder Protein ist, das die prokaryontische Zellmembran passieren kann.

5. Konjugat nach einem der Ansprüche 1 bis 4, wobei der Transportvermittler ein Phagen-Holin-Protein umfaßt, das eine der in Figur 3 dargestellten Aminosäuresequenzen umfaßt oder ein Fragment oder eine Variante davon, das (die) die prokaryontische Zellmembran passieren kann.

6. Konjugat nach einem der Ansprüche 1 bis 4, wobei der Transportvermittler ein Defensin umfaßt.

7. Konjugat nach einem der Ansprüche 1 bis 6, wobei die Peptid-Nukleinsäure (PNA) gegen ein Gen gerichtet ist, das eine Antibiotikum-Resistenz verleiht.

8. Konjugat nach Anspruch 7, wobei die Antibiotikum-Resistenz

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THE *myc* ONCOGENE: ITS ROLE IN TRANSFORMATION AND DIFFERENTIATION

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CONTENTS

INTRODUCTION

Much of the recent progress in understanding the origin of cancer has come from the study of cellular genes, termed "proto-oncogenes," that can induce unrestrained cell growth when mutated, incorporated within a retrovirus, or

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expressed inappropriately. One proto-oncogene, c-*myc*, has been the focus of numerous studies that have uncovered many of the major mechanisms by which normal cellular genes can be activated in tumor cells, including proviral insertion, gene amplification, and chromosomal translocations. Moreover, the expression of the c-*myc* gene is tightly linked to the action of growth factors and with the entry of cells into the cell cycle, suggesting that c-*myc* expression may be a major component in the regulatory networks associated with normal cell proliferation. The frequent involvement of c-*myc* in neoplastic transformation and its close association with cellular growth control have prompted an ever-expanding body of literature for a gene whose function within the cell is essentially unknown. In this review I describe some of the basic features of the c-*myc* locus and then focus on the role of the c-*myc* gene in cell proliferation and transformation. I do not discuss in detail the specific features of chromosomal rearrangements associated with c-*myc* activation, since this subject has been reviewed quite recently (24). I also refer the reader to other recent reviews on the general topic of oncogenes (11, 119).

THE c-*myc* GENE AND PROTEIN

*The c-*myc *Gene Structure*

The c-*myc* gene was first identified as the transforming sequence within the avian retrovirus MC29, and like many other proto-oncogenes, c-*myc* was found to be conserved in evolution (reviewed in 119). Early cloning studies identified two exons within the avian, mouse, and human cellular genes that were homologous to the viral sequences. However, subsequent sequence analysis of a murine c-*myc* cDNA (109) and human genomic clones (9, 10) identified one of the most intriguing features of the gene, namely a long untranslated first exon of 400–500 bp without initiation codons, but with termination codons, in all reading frames. The sequence of the first exon is 70% conserved between mouse and human (10) (whereas the coding exons are >90% homologous), but there is no significant conservation of first-exon sequences between chicken and mammals (71). The c-*myc* promoter is also somewhat unique; it has two distinct transcription start sites containing TATAA sequences separated by 160 bp, designated P1 and P2 (9, 10). Conservation of these features between avian and mammalian genes has prompted several models that propose an important regulatory role for the untranslated c-*myc* first exon (see below).

The c-*myc* gene is highly conserved among diverse chordates: it has been found in the genomes of humans, rodents, chickens, and trout (118). There is

REGULATION OF c-*myc* EXPRESSION

Transcriptional and Posttranscriptional Regulation

Determining what regulates the c-*myc* gene has become central to understanding the role of c-*myc* in cell transformation and in the response of cells to mitogens. Unlike the *ras* oncogenes, which are activated as the result of mutations within the protein-coding regions, no amino acid changes are required for c-*myc* activation. Therefore, attention has focused on quantitative differences in the levels of c-*myc* expression in tumor vs normal cells. Investigations into the factors and sequences that control steady-state levels of c-*myc* mRNA point to a complex pattern of regulation at both transcriptional and posttranscriptional levels.

The most important findings for understanding the regulation of the c-*myc* gene have been the connections between c-*myc* mRNA levels, mitogen stimulation, and the rate of cell proliferation. Kelly et al (59) showed that c-*myc* RNA levels are very low in quiescent, serum-starved fibroblasts and primary lymphocytes, and rapidly increase up to 40-fold in response to mitogens. In particular, c-*myc* RNA levels increase dramatically after treatment with platelet-derived growth factor, a mitogen that induces a state of "competence" in quiescent fibroblasts without promoting entry into S phase (90). Thus, c-*myc* induction has been associated with the entry of cells into the cell cycle, in particular, for the transition from a quiescent G_0 state into G_1. After an elevated transient induction by mitogens, mRNA in the stimulated cell returns to a level about 10-fold higher than that in quiescent cells. Initially, it had also been suggested that c-*myc* RNA levels might be modulated during the cell cycle, but it was subsequently shown that both RNA and protein levels are constant at all stages in growing cells (48, 117). Thus, the level of c-*myc* expression correlates with the rate of cell proliferation, suggesting that higher levels of c-*myc* may increase the probability of the cell's entry into S phase. In support of this hypothesis, investigators found that chicken fibroblasts transformed by MC29 viruses, which results in very high levels of *myc*, have a shorter cell-cycle time than normal cells or those transformed by other oncogenic viruses (85).

Expression of the c-*myc* gene appears to be regulated at both transcriptional and posttranscriptional levels. At the transcriptional level, serum stimulation of quiescent fibroblasts leads to a transient three- to fourfold increase in the c-*myc* transcription rate within two hours (47). However, no differences in transcription rate have been demonstrated for populations of dividing vs quiescent cells. Furthermore, the increased transcription rate observed during the initial phases of serum stimulation is not sufficient to account for the 20–40-fold increase in c-*myc* RNA levels (59). This observation suggests that

posttranscriptional control accounts for the remainder of the increase. Indeed, recent studies showed that c-*myc* RNA levels can be modulated in the absence of any change in the transcription rate of the gene (12). For example, both teratocarcinoma cells induced to differentiate and lymphoma cells treated with interferon suppressed their c-*myc* RNA level at least 20-fold, without any change in transcription rate (31, 34, 61). Thus, it appears that the primary mode of c-*myc* mRNA regulation in the transition from quiescent to proliferating cells (and vice versa) is posttranscriptional. Nevertheless, transcriptional control of the c-*myc* gene may be important at certain stages of cell differentiation as discussed below. Other important observations are that the amount of c-*myc* protein correlates directly with the RNA level (49) and that protein half-life does not change with growth rate (88).

Rapid Turnover of c-myc *mRNA*

Posttranscriptional modulation of c-*myc* mRNA levels could result either from altered mRNA transport or stability in the nucleus or from changes in cytoplasmic stability. Several lines of evidence suggest that cytoplasmic mRNA stability is the point at which regulation occurs. First, the half-life of c-*myc* mRNA is very short, from 10–30 min depending on the cell type (30). Furthermore, the superinduction of c-*myc* RNA observed when cycloheximide (along with mitogens) is added to cells is due to RNA stabilization (70). Thus, c-*myc* mRNA turns over very rapidly in the cytoplasm, and the rate of turnover is increased under conditions that suppress c-*myc* mRNA levels. The decrease in RNA turnover associated with cycloheximide treatment could result from a block in the synthesis of a labile sequence-specific RNase, or from an arrest in translation of c-*myc* mRNA, which in turn may be linked in some way to degradation.

Role of the First Exon in RNA Stability

More than one line of evidence suggests that the long 5' untranslated exon of the c-*myc* gene contributes to the short RNA half-life and to posttranscriptional control of RNA levels. Some differences in the half-life of RNAs with or without the first exon have been noted in comparisons of cell lines expressing truncated or normal genes (89, 93). However, the differences measured in plasmacytomas were not substantial (20–30 min for c-*myc* RNA containing the first exon and 60 min for those lacking it), and there was no comparison of different c-*myc* RNA structures in the same cell line. Recent experiments from our laboratory suggest a more complex picture, in which the first exon is required only for modulation of RNA levels in growth arrest, but not for mediation of the normal turnover of the short-lived message. Viral promoter–driven c-*myc* genes that lack the first exon have the same short half-life as the endogenous three-exon RNA in growing fibroblasts (G. D. Schuler & M. D.

Cole, manuscript in preparation). However, the half-life of the endogenous gene transcripts decreases during growth arrest in confluent, serum-starved cells, while the half-life of the transcripts that lack the first exon remains constant. Furthermore, transcripts lacking the first exon are not stabilized by cycloheximide (which superinduces the normal mRNA). Thus, the first exon may be the primary target for posttranscriptional modulation of RNA levels, but c-*myc* RNAs containing only the second and third exons still have a very short half-life. These results suggest the presence of a second region within the normal c-*myc* mRNA (in the 3' untranslated region or in the protein-coding region) that determines the rapid rate of RNA turnover.

The involvement of the first exon in modulation of RNA levels appears complex. Linkage of the c-*myc* promoter alone to a second gene, such as *neo*, does not confer the ability to modulate the gene by serum after transfection into fibroblasts (T. Jones, personal communication). Moreover, transcripts that contain virtually all of the first-exon sequences linked to *neo* are still not modulated by serum. Thus, the first exon appears to be necessary, but not sufficient, to modulate RNA-turnover rates in response to growth factors, and it does not appear to directly affect the short half-life of *myc* RNA.

MECHANISMS OF ONCOGENIC ACTIVATION

The c-*myc* proto-oncogene has been found to be "activated" by several different mechanisms that result in the unrestrained growth of tumor cells. The mechanisms of activation include proviral insertion, chromosomal translocation, and gene amplification. In fact historically, each of these mechanisms of proto-oncogene activation in tumor cells was first discovered through studies of the c-*myc* locus. Significantly, each of these mechanisms involves DNA rearrangements that lead to constitutive or elevated levels of c-*myc* expression, whereas, in contrast, few DNA rearrangements have been observed at the *ras* proto-oncogene loci. Thus, c-*myc* promotes tumor-cell growth via quantitative increases in protein level, rather than by qualitative changes in the protein, which are characteristic of *ras* activation. Despite intensive study, however, it is still unclear how each of these DNA rearrangements relates to the altered regulation and level of expression of the normal c-*myc* gene.

Levels of c-myc *Expression in Tumor vs Normal Cells*

Early studies with avian bursal lymphomas suggested that gene activation by retroviral insertion was associated with levels of c-*myc* RNA 10–100-fold higher than those in normal cells (50). However, subsequent studies of mouse plasmacytomas (PCs) and human Burkitt lymphomas (BLs) demonstrated that exceptionally high c-*myc* RNA levels are not always associated with DNA